Bioelectromagnetics 13:513–526 (1992)

Research on the Neurological Effects of Nonionizing Radiation at the University of Washington

Henry Lai

Department of Pharmacology and the Center for Bioengineering, University of Washington School of Medicine, Seattle

This paper reviews research on neurological effects of low-level microwave irradiation, which was performed at the University of Washington, during the decade of the 1980s. We studied in the rat the effects of microwave exposure on the actions of various psychoactive drugs, on the activity of cholinergic systems in the brain, and on the neural mechanisms involved. Our results indicate that endogenous opioids play an important mediating role in some of the neurological effects of microwaves, and that parameters of microwave exposure are important determinants of the outcome of the microwave effects.

Key words: low-level microwaves, psychoactive drugs, cholinergic systems, endogenous opioids, exposure parameters

INTRODUCTION

In honor of Dr. Guy's retirement, this paper was written as a review of the research carried out in the last decade at the Bioelectromagnetics Research Laboratory, Center for Bioengineering and the Department of Pharmacology, at the University of Washington, on the subject of neurological effects of nonionizing radiation.

My involvement began with a research project on the effects of drug-microwave interactions, which was sponsored by the Office of Naval Research. I became an investigator at the request of Dr. C.K. Chou, who at the time was with the University of Washington. The idea evolved as a result of information gleaned from the reports of Thomas and his co-workers [Thomas and Maitland, 1979; Thomas et al., 1979] on the interaction of low-level microwave irradiation with psychoactive drugs. The assumption is that the effects of microwaves can be better understood while an animal is under the influence of a drug.

Received for review October 31, 1991; revision received February 20, 1992.

Address reprint requests to Henry Lai, Ph.D., Department of Pharmacology, SJ-30, University of Washington, Seattle, WA 98195.

MICROWAVES AND ACTIONS OF PSYCHOACTIVE DRUGS

In this series of experiments, rats were irradiated in the circular waveguide system of Guy et al. [1979], and the actions of different psychoactive drugs on these animals were studied and compared with the responses of sham-exposed animals. The dependent variables studied were standard drug responses, such as change in body temperature, stereotypic behavior, and narcosis. These experiments yielded some interesting results. Different drug responses were affected in rats after acute (45-min) exposures to pulsed 2,450-MHz microwaves (1 mW/cm^2, whole body average SAR 0.6 W/kg, 2-μs pulses at 500 pps) including apomorphine-hypothermia, amphetamine-hyperthermia, ethanol-hypothermia, apomorphine-stereotypic behavior, morphine-catalepsy, the hypothermia and duration of narcosis induced by pentobarbital, and consumption of ethanol [Lai et al., 1983, 1984a,b]. An example of the effect of acute low-level microwave irradiation on ethanol-induced hypothermia in the rat is shown in Figure 1.

The effects of microwaves on drug actions were not unidirectional; that is, some drug effects (e.g., apomorphine-hypothermia and stereotypy) were enhanced by acute microwave exposure whereas others (e.g., ethanol-induced hypothermia and amphetamine-induced hyperthermia) were attenuated by irradiation. Therefore,

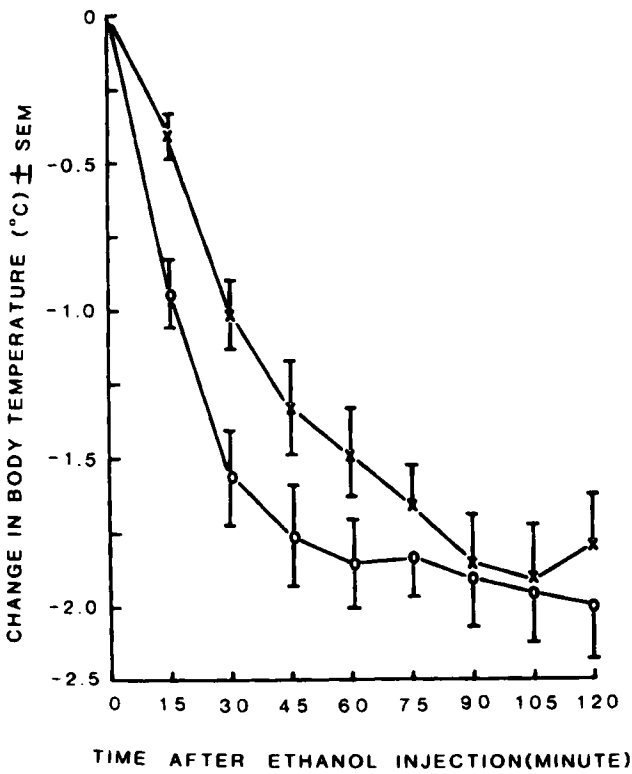

Fig. 1. Ethanol-induced hypothermia in microwave-treated (X) (n = 15) and sham-irradiated (0) (n = 14) rats. Rats were injected with 3 g/kg of ethanol intraperitoneally immediately after 45 min of microwave or sham exposure (time zero); colonic temperature was monitored at 15-min intervals. The thermal sequelae of the microwave-exposed rats were significantly different from those of the sham-exposed rats ($P < .05$). (Reproduced from Lai et al. [1984b]).

the effects could not be explained as a microwave-induced change in pharmacokinetics, such as change in permeability of the blood-brain barrier, distribution of the drug in the brain, or metabolism of the drugs.

Dennis Hjeresen, of the Los Alamos National Laboratory, confirmed the effect of microwaves on ethanol-induced hypothermia in the rat. Hjeresen used a similar exposure system and CW 2,450-MHz microwaves at a power density of 0.5 mW/cm^2 (whole-body average SAR 0.3 W/kg) [Hjeresen et al., 1988]. Blood ethanol levels indicated that the effect was not due to a change in metabolism or disposition of ethanol. Hjersen's subsequent experiments indicated the possibility of involvement of adrenergic systems in the brain [Hjeresen et al., 1989].

Further investigation in our laboratory showed that some of the effects of microwaves on drug actions could be classically conditioned to cues in the exposure environment [Lai et al., 1986b]. In classical conditioning, a biological response previously associated with one stimulus becomes associated with another through pairing. First, an unconditional response (UR) is elicited from an animal by an unconditional stimulus (US). After repeated pairing (conditioning) of the US with a neutral stimulus, which normally does not elicit the UR, presentation of the neutral stimulus alone (i.e., the conditional stimulus or CS) will elicit a conditional response (CR). Rats were exposed to a microwave US during ten, daily 45-min sessions. On the eleventh day, they were sham exposed and the effect of a psychoactive drug on these animals was studied. A CR was observed under the drug. Apparently, cues in the exposure environment became the CS.

Given these results, the question arose whether there is an underlying neural mechanism mediating the effects of microwaves on psychoactive drugs, or whether different mechanisms account for them. A clue was obtained from the finding that the effects of microwaves on amphetamine-hyperthermia could be blocked by pretreating the animals before exposure with the narcotic antagonist naloxone [Lai et al., 1986b]. Narcotic antagonists block the actions of endogenous opioids, which are a group of endogenous peptides in the brain, with opiate (e.g. morphine-like) activities and involved in various physiological functions, such as the stress-response, thermoregulation, and analgesia. Blockade of a biological effect by a narcotic antagonist implies that endogenous opioids play a role in the effect.

Further experiments showed that the effects of microwaves on apomorphine-induced hypothermia and ethanol-induced hypothermia also could be blocked by naloxone. Because the naloxone treatment by itself had no significant effect on the responses of sham-exposed animals, it was hypothesized that low-level microwaves activated the endogenous opioid systems (i.e., increased transmission) in the brain, which in turn altered the actions of the psychoactive drugs in the animal [Lai et al., 1987a]. This hypothesis is consistent with data from other pharmacological studies on the interactions between opiates and various psychoactive drugs. For example, it was found that acute, low-level microwave exposure enhanced apomorphine-induced hypothermia and stereotypy, consumption of ethanol, pentobarbital-induced narcosis and hypothermia, and morphine-induced catalepsy, but exposure attenuated amphetamine-induced hyperthermia and withdrawal from morphine. A survey of the literature showed that opiates had similar effects on these drug actions. The only exception was that microwaves attenuated the hypothermic effect of ethanol, whereas an enhancement of ethanol-induced hypothermia was generally observed when an animal was concomitantly injected with an opiate compound [cf. Table I in Lai et al., 1987a].

EFFECTS OF MICROWAVES ON CENTRAL CHOLINERIC SYSTEMS

When experiments on microwave-drug interactions were carried out, a series of studies on the effects of low-level microwaves on neurochemistry was performed. The effects of microwave exposure on cholinergic functions in the rat were studied with sodium-dependent high-affinity choline uptake (HACU) as an index of cholinergic activity [Atweh et al., 1975]. The cholinergic systems were studied because they are involved in many physiological and behavioral functions; on the other hand, the anatomy, neurochemistry, and pharmacology of the systems are well studied [Steriade and Biesold, 1990]. Deficits in cholinergic functions in the brain are also known to be related to various neurological and psychiatric disorders in human beings. For example, long-term changes in cholinergic activity after repeated stress might lead to the development of affective disorders such as anxiety and depression [Dilsaver, 1986; Janowsky et al., 1983], and loss of cholinergic innervation in the cerebral cortex and hippocampus has been suggested as the major cause of Alzheimer's syndrome [Davis and Maloney, 1976; Price et al., 1985].

Acute (45-min) exposures to pulsed (2 μs, 500 pps) microwaves in the circular waveguide (whole-body average SAR, 0.6 W/kg) reduced HACU in the frontal cortex and hippocampus of the rat. Furthermore, the effect on the hippocampus was blocked by narcotic antagonists, whereas that on the frontal cortex was not [Lai et al., 1987b]. These effects of microwaves were similar to those of stress from acute restraint [Lai et al., 1986c]. The restraint-stress-induced decrease in HACU in the hippocampus could be blocked by pretreatment with a narcotic antagonist, whereas its effect on frontal cortical HACU was insensitive to the drug treatment. Further research showed that, similar to the microwave-drug interactions, the effects of microwaves on central cholinergic activity could be classically conditioned to cues in the environment [Lai et al., 1987c].

Further experiments showed that low-level microwaves can exert a duration-dependent biphasic effect on cholinergic activity in the brain. After 20, instead of 45, minutes of irradiation (at average whole-body SAR of 0.6 W/kg), an increase in cholinergic activity was observed in the frontal cortex, hippocampus, and hypothalamus [Lai et al., 1989b], and these effects could be blocked by pretreatment with the narcotic antagonist naltrexone. The increase in cholinergic activity after a shorter duration of microwave exposure might explain the data of Monahan [1988]. Monahan studied effects of a 30-min exposure to CW 2,450-MHz microwaves (average whole-body SAR, 1 or 10 W/kg) in mice treated with cholinergic drugs. The response in tests of conditional suppression revealed enhanced activity of cholinergic systems.

The duration-dependent response to microwave irradiation reflect the dynamics of responses of the central cholinergic systems to perturbation. Duration- and intensity-dependent biphasic responses of the cholinergic systems to drugs and other treatments have been reported [Finkelstein et al., 1985; Lai, 1987; Vallano and McIntosh, 1980]. These results also indicate the importance of studying the contributory effects of different exposure parameters of microwaves.

PARAMETERS OF MICROWAVE EXPOSURE

Experiments were then carried out to investigate the effects of different parameters of microwave radiation on the cholinergic systems in the brain. In the first series of experiments, the effects of different exposure systems, thus different energy

absorption patterns in the body, and pulsed- vs. continuous-wave microwaves were compared. Rats were exposed to microwaves in the circular waveguide, or in the miniature anechoic chamber designed by Dr. A.W. Guy [Guy, 1979; Guy et al., 1979] with the whole-body average SAR kept at a constant level (0.6 W/kg). In the circular waveguide, rats were exposed to circularly polarized 2,450-MHz microwaves. In the miniature anechoic chamber, rats were exposed dorsally to plane-polarized microwaves. In both systems, the microwave source could be adjusted to produce either pulsed- (2 μs, 500 pps) or CW microwaves at different power densities. Detailed dosimetry studies of these two exposure systems were carried out [Chou et al., 1984, 1985a]; they showed that the circular waveguide produced a less uniform distribution of energy in the body of the exposed animal as compared with that occurring in the miniature anechoic chamber.

After 45 min of exposure to microwaves, a decrease in HACU (30–40%) was observed in the frontal cortex under all exposure conditions (circular waveguide vs. miniature anechoic chamber; pulsed vs. continuous waves). However, regardless of the exposure system used, hippocampal HACU was decreased after exposure to pulsed but not to CW microwaves. Striatal HACU was decreased after exposure to pulsed or CW microwaves in the miniature anechoic chamber, but no significant effect was observed when the animals were exposed in the circular waveguide. No significant effect on HACU was found in the hypothalamus under all the exposure conditions studied [Lai et al., 1988] (data are summarized in Figs. 2, 3). Thus, each brain region responded differently to microwave irradiation depending on exposure parameters. Effects on the

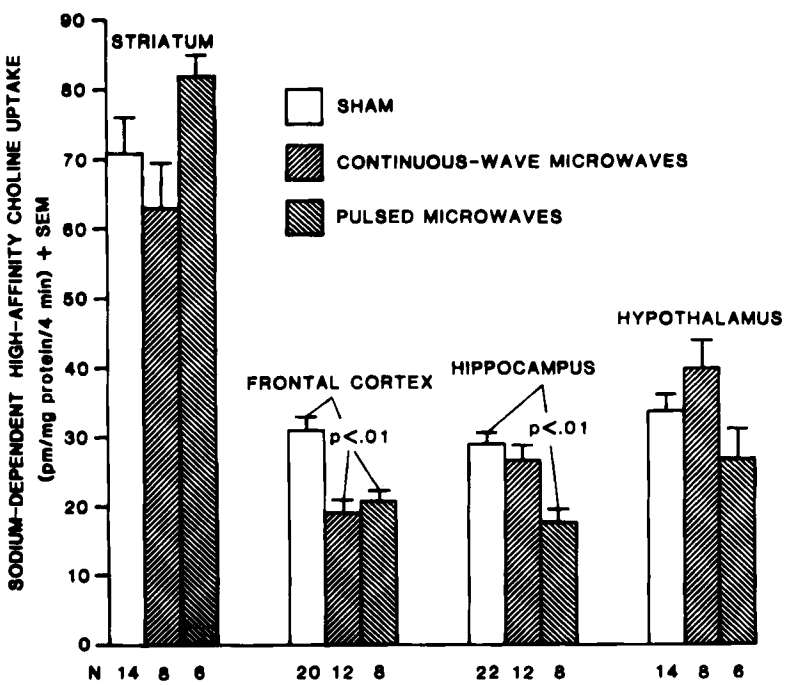

Fig. 2. Sodium-dependent high-affinity choline uptake in different areas of the rat brain after exposure to pulsed- or continuous-wave microwaves in the cylindrical waveguide. One-way analysis of variance showed significant treatment effects in the frontal cortex ($F[2,37 = 14.95, P < .005$), hippocampus ($F[2,40] = 10.65, P < .005$), and hypothalamus ($F[2,25] = 4.57, P < .025$). No significant treatment effect was observed in the striatum ($F[2,25] = 2.392$, N.S.). Differences between pairs of groups were evaluated by the Newman-Keuls test. (Reproduced from Lai et al. [1988]).

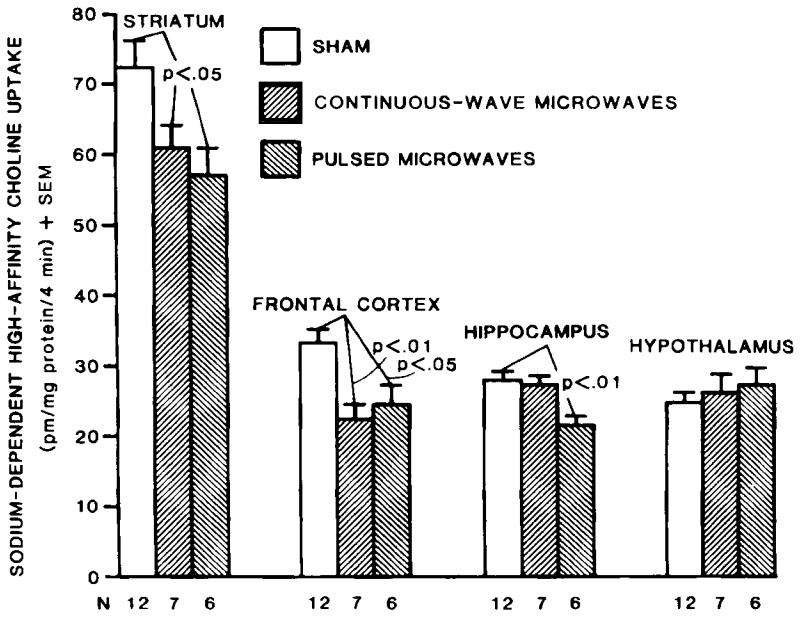

Fig. 3. Sodium-dependent high-affinity choline uptake in different areas of the rat brain after exposure to pulsed- or continuous-wave microwaves in a miniature anechoic chamber. One-way analysis of variance showed a significant treatment effect on the striatum ($F[2,22] = 3.692$, $P < .05$), frontal cortex ($F[2,22] = 7.236$, $P < .005$), and hippocampus ($F[2,22] = 7.315$, $P < .005$). No significant treatment effect was found for the hypothalamus ($F[2,22] = 0.895$, N.S.). Differences between paired groups were evaluated by the Newman-Keuls test. (Reproduced from Lai et al., [1988]).

frontal cortex were independent of the exposure system or use of pulsed or CW microwaves. The hippocampus responded to pulsed but not to CW microwaves. Response of the striatal cholinergic system depended on the exposure system used.

Another study was carried out to examine the dose-response relationship of microwave exposure on central cholinergic systems [Lai et al., 1989a]. High-affinity choline uptake in different regions of the brain was measured after acute (45-min) exposure to microwaves at different power densities in the circular waveguide. A dose-response function was observed (Fig. 4), and by use of probit analysis, the whole-body average SAR that elicited 50% of the maximal response (SAR_{50}), was obtained. SAR_{50} was calculated for each brain region. A higher dose-rate was required to elicit a response from the striatum (SAR_{50} = 0.65 W/kg), whereas the responses of the frontal cortex (SAR_{50} = 0.38 W/kg) and hippocampus (SAR_{50} = 0.44 W/kg) were similar. Thus, under the same irradiation conditions, different brain regions had different sensitivities to microwaves. The response characteristics of the striatal cholinergic system were of particular interest. In a dosimetry study [Chou

Fig. 4. Dose-response functions of sodium-dependent, high-affinity choline uptake after irradiation of rats with microwaves at different power densities and whole-body SARs in the striatum (**a**), frontal cortex (**b**), hippocampus (**c**), and hypothalamus (**d**). The * indicates a significant difference from corresponding values of sham-irradiated controls at $P < .01$ (Newman-Keuls test). P.D. = power density in mW/cm^2; SAR = average whole-body specific absorption rate in W/kg; SEM = standard error of the mean; N = sample size. (Reproduced from Lai et al. [1989a]).

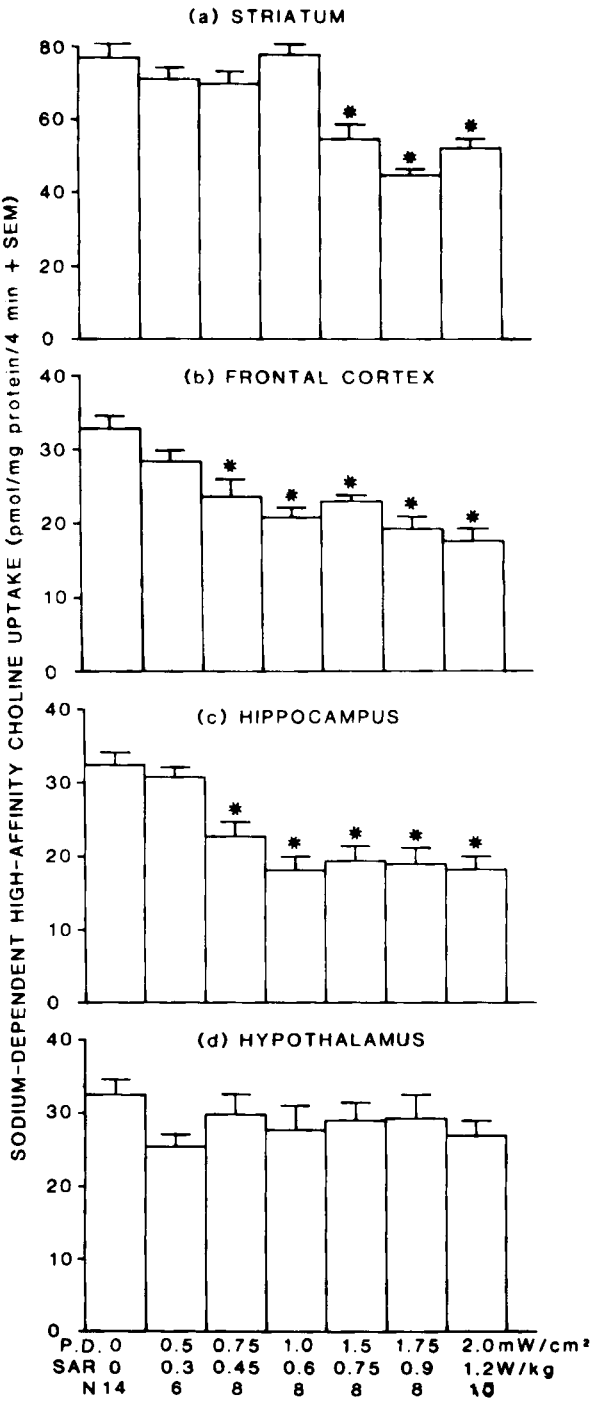
(a) STRIATUM
(b) FRONTAL CORTEX
(c) HIPPOCAMPUS
(d) HYPOTHALAMUS
SODIUM-DEPENDENT HIGH-AFFINITY CHOLINE UPTAKE (pmol/mg protein/4 min + SEM)
0
20
40
60
80
10
30
P.D. 0 0.5 0.75 1.0 1.5 1.75 2.0mW/cm²
SAR 0 0.3 0.45 0.6 0.75 0.9 1.2W/kg
N 14 6 8 8 8 8 10

Fig 4.

et al., 1985a], the rate of energy absorption in the striatum was about five times higher when a rat was exposed in the circular waveguide than when it was exposed in the miniature anechoic chamber. However, a change in HACU was observed in the striatum at a lower average whole-body SAR when the animal was being exposed in the miniature anechoic chamber as compared with the circular waveguide. Thus, the effect observed was not directly related to the localized rate of energy absorption in the striatum.

CHANGES IN THE CHOLINERIC SYSTEMS AFTER REPEATED MICROWAVE EXPOSURE

Further experiments were conducted to investigate the effects of repeated microwave exposure on the cholinergic systems. Changes in muscarinic cholinergic receptors were investigated because these receptors are known to change their properties after repeated perturbations of the cholinergic system, and because such changes are important in an animal's normal physiological functions [Overstreet and Yamamura, 1979]. Changes in receptors could presumably last longer (days to weeks) after termination of treatment than changes in HACU (hours). After ten daily sessions of microwave exposures (at average whole-body SAR of 0.6 W/kg), the concentration of muscarinic cholinergic receptors changed in the brain [Lai et al., 1989b]. However, the direction of change depended on the acute effect of microwaves. When animals were given daily sessions of a 20-min exposure, which increased cholinergic activity in the brain, a decrease in the concentration of receptors was observed. On the other hand, when animals were subjected to daily 45-min exposure sessions, which decreased cholinergic activity in the brain, an increase in the concentration of cholinergic receptors resulted after repeated exposure. These data led to an important conclusion that the long-term biological consequence of repeated microwave irradiation depend, also, on parameters of irradiation. Further experiments showed that changes in cholinergic receptors, after repeated microwave exposure, also depended on endogenous opioids in the brain, because the effects could be blocked by pretreatment before each session of daily exposure with the narcotic antagonist naltrexone [Lai et al., 1991].

NEURAL MECHANISMS MEDIATING THE EFFECT OF MICROWAVES ON THE CHOLINERIC SYSTEMS

Our experiments have shown that endogenous opioids in the brain mediate the effects of low-level microwaves on the cholinergic systems in the brain. Because endogenous opioids affect brain functions by acting on different subtypes of receptors, to further elucidate the neurological effects of microwaves, experiments were conducted to investigate the subtypes of opioid receptors in the brain that mediate effects of microwave-induced decreases in cholinergic activity. Three main subtypes of opioid receptors, μ, δ, and κ, were found in the brain [Mansour et al., 1987; Katoh et al., 1990].

We found that the effect of an acute microwave exposure (45 min at average whole-body SAR of 0.6 W/kg) on hippocampal HACU could be blocked by injection of specific μ, δ, or κ opioid antagonists into the lateral cerebroventricle before microwave exposure. However, supporting the previous finding that the

microwave-induced decrease in cholinergic activity in the frontal cortex was not blocked by narcotic antagonists, all types of opioid receptor antagonists tested were not effective in blocking the microwave-induced decrease in HACU in the frontal cortex [Lai et al., 1992b].

More recent experiments have shown that the effects of microwaves on both frontal cortical and hippocampal cholinergic systems can be blocked by pretreatment with an intracerebroventricular injection of the corticotropin-releasing factor (CRF) antagonist α-helical-CRF_{9-41} [Lai et al., 1990]. From the findings described above and the results of other research [Lai and Carino, 1990], showing that the effect of CRF on hippocampal HACU is mediated by endogenous opioids, the following model on the sequence of events in the brain triggered by microwaves was proposed (Fig. 5): microwaves (45-min exposure at average whole-body SAR of 0.6 W/kg) activate CRF in the brain, which in turn causes a decrease in activity of the cholinergic innervations in the frontal cortex and hippocampus of the rat. In addition, the effect of CRF on hippocampal cholinergic system is mediated by endogenous opioids via μ, δ, and κ opioid receptors. However, it is not known how microwaves activate CRF and endogenous opioids in the brain. Also unknown are the pathways in the brain that mediate these effects of microwaves. It is possible that other neurotransmitters in the brain might play mediating roles in this model.

OTHER NEUROLOGICAL STUDIES

Because cholinergic systems in the brain are involved in learning and memory functions, the effects of microwaves on performance in the radial-arm-maze were studied. This is a behavioral task requiring spatial memory and known to involve both frontal cortical and hippocampal cholinergic functions [Levin, 1988]. After they were exposed to microwaves, which decreased cholinergic activity in the brain (45 min at average whole-body SAR of 0.6 W/kg), rats showed a learning deficit [Lai et al., 1989b]. The animals made significantly more errors during training sessions and learned more slowly than sham-exposed rats.

We have speculated that low-level microwave irradiation is a "stressor" [Lai et al., 1987a]. The findings that CRF and endogenous opioids are activated (i.e., increased transmission) by microwaves support his notion, because both of these neurotransmitter/modulator(s) are known to be involved in stress responses [Amir et al., 1980; Fisher, 1989]. To further explore the hypothesis that relatively weak microwave fields may cause stress responses, a series of experiments were performed to study their effect on benzodiazepine receptors in the rat brain. Benzodiazepine

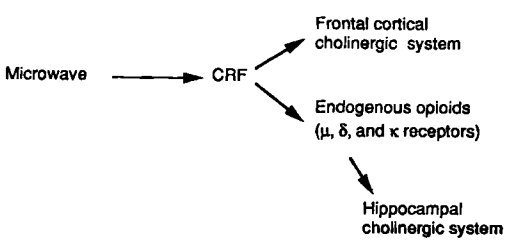

Fig. 5. A model of neural mechanisms mediating the effects of low-level microwaves on the activity of the cholinergic innervations in the frontal cortex and hippocampus of the rat.

receptors are believed to be involved in anxiety and stress responses in animals, and they have been shown to change after acute or repeated exposure to various sources of stress [Braestrup et al., 1979; Medina et al., 1983]. After an acute (45-min) exposure to microwaves in the circular waveguide (at average whole-body SAR of 0. 6 W/kg), an increase in the concentration of benzodiazepine receptors occurred in the cerebral cortex, but no significant effect was observed in the hippocampus and cerebellum. The response of the cerebral cortex exhibited adaptation after repeated exposure (ten 45-min sessions) to microwaves [Lai et al., 1992a]. Microwave-induced change in benzodiazepine receptors in the brain might explain the results of Thomas and his co-workers [Thomas and Maitland, 1979; Thomas et al., 1979] on the interaction of microwaves and benzodiazepine drugs on operant behavior, and also the data from two earlier studies showing changes in benzodiazepine-related functions in the rat after exposure to microwaves in the circular waveguide [Hjeresen et al., 1987; Johnson et al., 1982].

With the shift of research emphasis on the biological effects of ELF electromagnetic radiation, recent experiments were designed to study the effects of a 60-Hz magnetic field on the cholinergic systems of the brain of the rat. A dose-response study was carried out. After 45 min of exposure to the magnetic field at flux densities greater than 0.75 mT, a decrease in HACU was observed in both the frontal cortex and hippocampus. Moreover, the effects were blocked by pretreatment with naltrexone, but not the peripheral narcotic antagonist naloxone methiodide [Lai et al., 1993]. These data indicate that magnetic-field-induced decreases in HACU may be mediated by endogenous opioids located in the central nervous system. Unlike the finding with microwaves, the magnetic-field-induced decrease in frontal cortical cholinergic activity was also mediated by endogenous opioids, which indicate that the two types of electromagnetic fields may trigger different neural mechanisms.

SUMMARY AND DISCUSSION

Several conclusions and speculations could be made from our data on the effects of low-level microwave irradiation on the central nervous system. An important conclusion is that endogenous opioids may play a mediating role in some of the neurological effects of microwaves that we studied. Our research data supporting the role of endogenous opioids in biological effects of microwaves could be summarized as follows: 1) microwaves enhanced morphine-induced catalepsy in the rat [Lai et al., 1983]; 2) microwaves attenuated the naloxone-induced wet-dog shake, a morphine withdrawal symptom, in morphine-dependent rats [Lai et al., 1986a]; 3) narcotic antagonist blocked a transient increase in body temperature after microwave exposure [Lai et al., 1984c]; 4) the effect of acute microwave exposure on amphetamine-induced hyperthermia [Lai et al., 1986b], ethanol-induced hypothermia, and apomorphine-induced hypothermia [unpublished results] can be blocked by narcotic antagonist; 5) microwave-induced changes in HACU in the brain can be blocked by narcotic antagonists [Lai et al., 1987b, 1989b]; 6) changes in concentrations of muscarinic cholinergic receptors in the brain after repeated sessions of microwave exposure can be blocked by pretreatment with narcotic antagonists before each session of microwave exposure [Lai et al., 1991]; 7) more recently, by using specific opioid receptor antagonists, we found that the three major subtypes of opioid receptors, μ, δ, and κ, are involved in the effect of microwaves on hippocampal HACU [Lai et al., 1992b]. In these studies, treatment with the narcotic

antagonists in sham-exposed animals had no significant effect on the responses studied, indicating that endogenous opioid release was phasic and triggered by microwave irradiation.

Parameters of the microwave irradiation are an important consideration in the production of biological effects. Different durations of acute exposure lead to different biological effects and, consequently, different long-term effects occur after repeated exposure. The waveform of the radiation is also important. This was seen in the differential effects that occurred after exposure to pulsed- vs. continuous-wave microwaves, plane vs. circularly polarized waves, and the pattern of energy absorption in the body. These findings raise the question whether the whole-body average SAR can be used as the only determining factor in evaluating biological effects of low-level microwaves. Other features of the radiation also should be considered. For example, an effect on hippocampal HACU was due to a specific property of pulsed microwaves, since it was affected by pulsed and not by CW irradiation and also was independent of the exposure system used. A possible mechanism of effect was the auditory effect of pulsed microwaves [Chou et al., 1982; Lin, 1978]. The whole-body dose of energy per pulse, when a decrease in hippocampal HACU was observed (circular waveguide: 0.9–2.4 mJ/kg; miniature anechoic chamber: 1.2 mJ/kg), was within the threshold of the auditory response of rats (0.91–1.8 mJ/kg whole-body average energy absorption per pulse) as reported by Chou et al. [1985b].

Another interesting finding was that the areas of the brain that show changes in cholinergic activity did not correlate with localized SARs in the brain. A detailed study on localized SARs in different brain areas of rats exposed to microwaves in the circular waveguide and miniature anechoic chamber had been carried out [Chou et al., 1985b]. In this dosimetry study, a rat carcass was exposed to microwaves in the circular waveguide with its head either facing toward (anterior exposure) or away from (posterior exposure) the source, and in the miniature anechoic chamber with the axis of the body parallel to either the E or H field. These positions were studied because, during in vivo exposures, an unrestrained rat spent most of its time in these orientations (i.e., with its body parallel to the long axis of the holding cage).

The dosimetry data showed that the septum, where the cell bodies of the hippocampal cholinergic innervations are located, had the lowest local SAR among the eight brain areas measured in all the positions studied in both exposure systems. However, the hippocampal cholinergic system responded to pulsed, but not continuous-wave, microwave irradiation in both exposure systems. In the striatum, the local SAR was approximately five times higher when the animals were exposed in the circular waveguide (average of anterior and posterior exposure) than in the miniature anechoic chamber (body parallel to E field, which was used in the in vivo study). However, the striatal cholinergic system responded when the animal was exposed in the miniature anechoic chamber, but not in the circular waveguide. Because the cholinergic terminals in the striatum are mostly from interneurons inside that brain structure, this result would provide an ample argument against a direct-action effect on the striatal cholinergic neurons by microwaves.

The cholinergic innervation in the frontal cortex (cortex above the olfactory tubercle) of the rat responded to all conditions of exposure (irrespective of the exposure system or whether the microwaves were pulsed or continuous-wave), which produced a wide range of local SARs in that area of the brain (0.11–1.85 W/kg per mW/cm^2). Yet, in both exposure systems, the extent of the neurochemical response was similar (30–40% decrease in HACU).

Unless different brain areas have different sensitivity to the direct effect of microwaves, we would conclude that the effects of microwaves on cholinergic activity in the brain regions originated from other sites in the brain or body. To trace the pathways of effects, further experiments should be carried out. In addition, speculation could be made that local heating was not responsible for the changes in cholinergic activity observed in the different brain areas. Certainly, this would also depend upon the efficiency of heat dissipation (e.g., local blood supply and flow) in each brain area.

We speculated that low-level microwave irradiation is a "stressor" [Lai et al., 1987a]. This hypothesis is based on the similarity of the effects of microwaves and those of established sources of stress. Recent findings that microwaves can activate endogenous opioids and CRF and can affect benzodiazepine receptors in the brain also support the hypothesis, because these neural mechanisms are known to be involved in stress responses [Amir et al., 1980; Fisher, 1989; Polc, 1988]. However, it is difficult to prove that a particular entity is a source of stress. If stress is defined as a perturbation of homeostasis, and repeated perturbation leads to changes in the normal functions of an organism, then microwaves will fit into that category. Changes in central cholinergic activity and deficits in spatial learning after a single exposure are signs of perturbation of normal functions. The classically conditioned effects and changes in receptors after repeated exposure indicate that long-term changes occur in response to radiation. These changes could be compensatory changes in response to the perturbation. On the other hand, with the changes in receptor properties, normal physiological and behavioral functions would be altered, and an animal would respond differently to further challenges by the radiation or by other factors in the environment. Some of these altered responses might be maladaptive. However, a criterion of stress is that detrimental changes and a breakdown of the response system occur after prolonged or repeated exposure. At present, there is no convincing evidence that repeated exposure to low-level microwaves could lead to irreversible neurological effects. Perhaps a longer or more frequent exposure is necessary to produce such effects.

ACKNOWLEDGMENTS

Research described in this paper was supported by the Office of Naval Research (contract N0014-80-C-0354) and the National Institute of Environmental Health Sciences (ES-03712).

REFERENCES

Amir S, Brown ZW, Amit Z (1980): The role of endorphins in stress: Evidence and speculations. Neurosci Biobehav Rev 4:77–86.

Atweh S, Simon JR, Kuhar MJ (1975): Utilization of the sodium-dependent high-affinity choline uptake in vitro as a measure of activity of cholinergic neurons in vivo. Life Sci 17:1535–1544.

Braestrup C, Neilsen M, Neilsen EB, Lyon M (1979): Benzodiazepine receptors in the brain as affected by different experimental stresses: The changes are small and not unidirectional. Psychopharmacology 65:273–277.

Chou CK, Guy AW, Galambos R (1982): Auditory perception of radiofrequency electromagnetic fields. J Acoust Soc Am 71:1321–1334.

Chou CK, Guy AW, Johnson RB (1984): SAR in rats exposed in 2450-MHz circularly polarized waveguide. Bioelectromagnetics 5:389–398.

Chou CK, Guy AW, McDougall J, Lai H (1985a): Specific absorption rate in rats exposed to 2450-MHz microwaves under seven exposure conditions. Bioelectromagnetics 6:73–88.

Chou CK, Yee KC, Guy AW (1985b): Auditory response in rats exposed to 2450-MHz electromagnetic fields in circularly polarized waveguide. Bioelectromagnetics 6:323–326.

Davies P, Maloney AJF (1976): Selective loss of central cholinergic neurons in Alzheimer's disease. Lancet 2:403.

Dilsaver SC (1986): Pathophysiology of "cholinoceptor supersensitivity" in affective disorder. Biol Psychiatry 21:813–829.

Finkelstein Y, Koffler B, Rabey JM, Gilad GM (1985): Dynamics of cholinergic synaptic mechanisms in rat hippocampus after stress. Brain Res 343:314–319.

Fisher LA (1989): Corticotropin-releasing factor: Endocrine and autonomic integration of responses to stress. Trends Pharmacol Sci 10:189–193.

Guy AW (1979): Miniature anechoic chamber for chronic exposure of small animals to plane wave microwave field. J Microwave Power 14:327–338.

Guy AW, Wallace J, McDougall JA (1979): Circularly polarized 2450-MHz waveguide system for chronic exposure of small animals to microwaves. Radio Sci 14(6s):63–74.

Hjeresen DL, Francendese A, O'Donnell JM (1988): Microwave attenuation of ethanol-induced hypothermia: Ethanol tolerance, time course, exposure duration and dose response studies. Bioelectromagnetics 9:63–78.

Hjeresen DL, Francendese A, O'Donnell JM (1989): Microwave attenuation of ethanol-induced interactions with noradrenergic neurotransmitter systems. Health Phys 56:767–776.

Hjeresen DL, Umbarger KO, McElroy JF (1987): Benzodiazepine receptor antagonist R015-1788 blocks the 2.45 GHZ microwave attenuation of ethanol-induced hypothermia. In Kleinstein BH Jr, Goldberg RB, Collier MN eds: "Biological Effects of Nonionizing Electromagnetic Radiation: A Digest of Current Literature," Vol. XI, No. 1. Philadelphia: Information Ventures Inc, p 56.

Janowsky DS, Risch SC, Huey L, Judd LL, Rausch J (1983): Central physostigmine induced cardiovascular and behavioral changes: Toward an acetylcholine hypothesis of stress. Psychopharmacol Bull 19:675–681.

Johnson RB, Hamilton J, Chou CK, Guy AW (1982): Pulsed microwave reduction of diazepam-induced sleeping in the rat. In Kleinstein BH Jr (Project Manager): "Biological Effects of Nonionizing Radiation: A Digest of Current Literature," Vol. V, No. 2–4 (NTIA-fjH-82-16). Philadelphia Science Information Inc., p 89.

Katoh A, Nabeshima T, Kameyama T (1990): Behavioral changes induced by stressful situation: Effects of enkephalins, dynorphin, and their interaction. J Pharmacol Exp Ther 253:600–607.

Lai H (1987): Acute exposure to noise affects sodium-dependent high-affinity choline uptake in the central nervous system of the rat. Pharmacol Biochem Behav 28:147–151.

Lai H, Carino MA (1990): Effects of noise on high-affinity choline uptake in the frontal cortex and hippocampus of the rat are blocked by intracerebroventricular injection of corticotropin-releasing factor antagonist. Brain Res 527:354–358.

Lai H, Carino MA, Horita A, Guy AW (1989a): Acute low-level microwave exposure and central cholinergic activity: A dose-response study. Bioelectromagnetics 10:203–209.

Lai H, Carino MA, Horita A, Guy AW (1989b): Low-level microwave irradiation and central cholinergic systems. Pharmacol Biochem Behav 33:131–138.

Lai H, Carino MA, Horita A, Guy AW (1990): Corticotropin-releasing factor antagonist blocks microwave-induced changes in central cholinergic activity in the rat. Brain Res Bull 25:609–612.

Lai H, Carino MA, Horita A, Guy AW (1992a): Single vs. repeated microwave exposure: Effects on benzodiazepine receptors in the brain of the rat. Bioelectromagnetics 13:57–66.

Lai H, Carino MA, Horita A, Guy AW (1992b): Opioid receptor subtypes that mediate a microwave-induced decrease in central cholinergic activity in the rat. Bioelectromagnetics 13:237–246.

Lai H, Carino MA, Horita A, Guy AW (1993): Effects of a 60Hz magnetic field on central cholinergic systems of the rat. Bioelectromagnetics (In press).

Lai H, Carino MA, Wen YF, Horita A, Guy AW (1991): Naltrexone pretreatment blocks microwave-induced changes in central cholinergic receptors. Bioelectromagnetics 12:27–33.

Lai H, Horita A, Chou CK, Guy AW (1983): Psychoactive drug response is affected by acute low-level microwave irradiation. Bioelectromagnetics 4:205–214.

Lai H, Horita A, Chou CK, Guy AW (1984a): Acute low-level microwave irradiation and the actions of pentobarbital: Effects of exposure orientation. Bioelectromagnetics 5:203–212.

Lai H, Horita A, Chou CK, Guy AW (1984b): Low-level microwave irradiation affects ethanol-induced hypothermia and ethanol consumption. Bioelectromagnetics 5:213–220.

Lai H, Horita A, Chou CK, Guy AW (1984c): Microwave-induced postexposure hyperthermia: Involvement of endogenous opioids and serotonin. IEEE Tran Microwave Th Tech MTT-32:882–886.

Lai H, Horita A, Chou CK, Guy AW (1986a): Low-level microwave irradiation attenuates naloxone-induced withdrawal syndrome in morphine-dependent rats. Pharmacol Biochem Behav 24:151–153.

Lai H, Horita A, Chou CK, Guy AW (1986b): Effects of low-level microwave irradiation on amphetamine hyperthermia are blockable by naloxone and classically conditionable. Psychopharmacology 88:354–361.

Lai H, Horita A, Chou CK, Guy AW (1987a): A review of microwave irradiation and actions of psychoactive drugs. IEEE Engin Med Biol 6:31–36.

Lai H, Horita A, Chou CK, Guy AW (1987b): Low-level microwave irradiation affects central cholinergic activity in the rat. J Neurochem 48:40–45.

Lai H, Horita A, Chou CK, Guy AW (1987c): Effects of low-level microwave irradiation on hippocampal and frontal cortical choline uptake are classically conditionable. Pharmacol Biochem Behav 27:635–639.

Lai H, Horita A, Guy AW (1988): Acute low-level microwave exposure and central cholinergic activity: Studies on irradiation parameters. Bioelectromagnetics 9:355–362.

Lai H, Zabawska J, Horita A (1986c): Sodium-dependent, high-affinity choline uptake in hippocampus and frontal cortex of the rat affected by acute restraint stress. Brain Res 372:366–369.

Levin ED (1988): Psychopharmacological effects in the radial-arm maze. Neurosci Biobehav Rev 12:169–175.

Lin JC (1978): "Microwave Auditory Effects and Applications." Springfield, IL: Charles C. Thomas.

Mansour A, Khachaturian H, Lewis ME, Akil H, Watson SJ (1987): Autoradiographic differentiation of mu, delta, and kappa opioid receptors in the rat forebrain. J Neurosci 7:2445–2464.

Medina JH, Novas ML, Wolfman CNV, Levi DeStein M, De Robertis E (1983): Benzodiazepine receptors in rat cerebral cortex and hippocampus undergo rapid and reversible changes after acute stress. Neuroscience 9:331–335.

Monahan JC (1988): Microwave-drug interactions in the cholinergic nervous system of the mouse. In O'Connor ME, Lovely DH (eds): "Electromagnetic Fields and Neurobehavioral Function." New York: Alan R. Liss Inc., pp 309–326.

Overstreet DH, Yamamura H (1979): Receptor alteration and drug tolerance. Life Sci 25:1865–1878.

Polc P (1988): Electrophysiology of benzodiazepine receptor ligands: Multiple mechanisms and sites of action. Prog Neurobiol 31:349–424.

Price DL, Cork LC, Struble RG, Whitehouse PJ, Kitt CA, Walker LC (1985): The functional organization of the basal forebrain cholinergic system in primates and the role of the system in Alzheimer's disease. Ann N Y Acad Sci 444:287–295.

Steriade M, Biesold D (1990): "Brain Cholinergic Systems." Oxford: Oxford University Press.

Thomas JR, Burch LS, Yeandle SC (1979): Microwave radiation and chlordiazepoxide: Synergistic effects on fixed interval behavior. Science 203:1357–1358.

Thomas JR, Maitland G (1979): Microwave radiation and dextroamphetamine: evidence of combined effects on behavior of rats. Radio Sci 14:253–258.

Vallano ML, McIntosh TK (1980): Morphine stimulates cholinergic neuronal activity in the rat hippocampus. Neuropharmacology 19:851–853.